we find that Hsp90 mutants deficient for ATP binding or hydrolysis have differential impacts on the activation of kinase and hormone receptor clients in vivo. These results provide a rationale for understanding anti-cancer drugs that competitively bind to the ATPase site of Hsp90.

Heme Proteins

2240-Pos Board B210

Reactive Vibrational Dynamics of Iron in Heme

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Nuclear resonance vibrational spectroscopy (NRVS) measurements supported by DFT calculations identify vibrational modes of the iron atom in halide derivatives of iron porphyrins. These compounds capture many essential aspects of heme geometry and vibrations. The smaller (porphine) models simplify the vibrational spectrum and enable accurate analysis using DFT methods. NRVS identifies both doming and Fe-halide stretching components of the reaction coordinate with confidence. Correlation analysis between 4-coordinate and 5-coordinate compounds suggests significant mixing between Fe-ligand and heme modes. Measurements and calculations on larger porphyrins reveal the effect of peripheral groups. Measurements on oriented porphine halide crystals definitively identify the contribution of in plane and out of plane Fe motion. The frequency of heme doming vibrations varies in a systematic manner with peripheral substitution and halide mass, which will allow us to evaluate their contributions to vibrational signals that follow femtosecond laser excitation. Such measurements will ultimately enable quantitative estimates of the energetics of molecular distortions that modulate reaction rates in heme proteins.

2241-Pos Board B211

Low frequency dynamics of Cystathionine beta-synthase

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Femtosecond coherence spectroscopy is used to study the low frequency dynamics of cystathionine beta-synthase (CBS). CBS is a pyridoxal-5′-phosphate-dependent heme enzyme with cysteine and histidine axial ligands that catalyzes the condensation of serine and homocysteine to form cystathionine. Resonance excitation near the maximum of the ferric state Soret band reveals a mode near ~40 cm^{-1} (phase ~pi/2). The phase indicates that the initial non-equilibrium coherent wavepacket for this mode is dominated by a momentum displacement. This is consistent with doming of the ferric five-coordinate species and suggests photolysis of the histidine ligand. When exciting on the red side of the Soret band, a mode near ~25 cm^{-1} is observed that exhibits a phase jump of ~pi for blue-side excitation. This mode may involve the response of an unphotolyzed fraction of hot ferric six-coordinate species, subsequent to ultrafast non-radiative decay. A strong correlation between the ''detuned'' coherence spectrum (which reveals higher frequencies) and the Raman spectrum is also demonstrated. Normal coordinate structural decomposition of the ferric heme crystal structure predicts strong saddling, doming, and ruffling modes and they are observed in the coherence spectra. The relative intensities of these modes are monitored as a function of pH in order to explore the potential correlation between redox equilibria, pH, and protein-induced heme structural perturbations. The low frequency spectra of ferrous CBS and its NO-bound complex were also obtained, along with the CO rebinding kinetics. The geminate rebinding of CO to CBS was found to be unusually fast and similar to that of CooA.

2242-Pos Board B212

Proximal Ligand Switch Triggered by Carbon Monoxide in Inducible Nitric Oxide Synthase

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Inducible Nitric Oxide Synthase (iNOS) is one of three isoforms of NOS, responsible for the oxidation of L-Arginine to L-citrulline and nitric oxide (NO). This isoform is implicated in the pathophysiology of several inflammatory disorders including arthritis, atherosclerosis, and transplant rejection. iNOS is unique among the isoforms in that it is not regulated by the intracellular calcium concentration. Instead, iNOS is exclusively regulated at the transcriptional and molecular levels. One molecular regulator of iNOS is Carbon Monoxide (CO) generated by heme oxygenase (HO-1), an inducible enzyme that produces CO and is known to mediate anti-inflammatory effects. It has been shown that CO binding to iNOS promotes its gradual conversion to an inactive ''p420'' form. On this basis, we hypothesize that the cross-talk between HO-1 and iNOS plays an important role in attenuating the activity of iNOS and modulating inflammatory responses in vivo. The structure of the iNOS p420 has been a subject of debate, as the proximal ligand has been proposed to be either a histidine residue or a protonated, neutral thiol form of the native cysteine thiolate. In this work, we use resonance Raman Spectroscopy to explore the properties of the p420 derivative of iNOS in order to resolve this issue. We show that the nanosecond time-resolved Raman spectrum of iNOS p420 exhibits a band consistent with an iron-histidine stretching mode. To evaluate the identity of the proximal ligand of iNOS p420, we measured the Raman spectra of the H93G cavity mutant and organic model compounds with a neutral thiol coordinated to them as reference systems. On the basis of these studies, we postulate a novel reversible ligand-switching mechanism that may be critical for the in vivo regulation of iNOS activity involving endogenous CO.

2243-Pos Board B213

Resonance Raman Investigation of the R481 Mutants of Cytochrome *c* Oxidase from *R. sphaeroides*

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The enzymes of the heme-copper oxidase superfamily have a highly conserved arginine residue (R438, R481, and R473 of the bovine, *R. sphaeroides*, and *P. denitrificans* cytochrome *c* oxidases, respectively), which is located close to the heme-propionate substituents of the heme *a* and heme a_3 moieties. To explore the structural and functional implications of this conserved arginine, we used resonance Raman spectroscopy to study the heme vibrational spectra of the R481 mutant proteins (R481H, R481Q, and R481L) of cytochrome *c* oxidase from *R. sphaeroides*. All the mutants showed significant down-shifts in the C=O stretching vibrational frequencies of the heme *a* and a_3 formyl substituent groups in the fully oxidized state, while they showed up- and down-shifts of the *a* and a_3 formyl C=O stretching modes, respectively, in the fully reduced state. On the basis of these observations, the role of the conserved arginine will be discussed.

2244-Pos Board B214

Indoleamine 2, 3- Dioxygenases 2: The Missing Link For The 1-methyl-D-trp Mechanism Of Action?

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Indoleamine 2, 3-dioxygenase (IDO1) is one of the only two heme-containing enzymes that catalyze the first and rate-limiting step of the kynurenine pathway of L-Trp metabolism. IDO1 has been implicated in the escape mechanism of cancer cells from immune surveillance. Consequently, IDO1 has been recognized as an important anticancer drug target. Recent studies showed that an IDO1 inhibitor, 1-methyl-Trp (1-M-Trp), triggers antitumor immunity and can be used to improve the efficacy of traditional chemotherapeutic drugs. Preclinical studies showed that the D stereoisomer of 1-M-Trp exhibits superior antitumor activity; however, it is less potent for the purified enzyme. On this basis, a second isoform of IDO1 had been suspected. It was not until last year that this second isoform of IDO1, named IDO2, was identified. To understand the missing link for the D-1-M-Trp mechanism of action, we have cloned, expressed and purified recombinant human IDO2. Resonance Raman and optical absorption spectroscopic studies showed that IDO2 exhibits structural features slightly different from IDO1. Furthermore, an activity assay with D- or L-Trp shows that IDO2 does not produce N'-Formyl-kynurenine as IDO1 does; instead, a new product with an absorption maximum at 344 nm was produced. Stopped-flow measurements show that IDO2 binds O2 in a similar fashion as IDO1. Together these data indicate novel function and action mechanisms of IDO2 that are distinctive from IDO1.

2245-Pos Board B215

Linking Heme Activation to Conformation Change in Hemoglobin Via Chain Selective Time-resolved Resonance Raman Spectroscopy on Mesoheme Hybrids

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Time-resolved Resonance Raman spectra are reported for Hb tetramers, in which the α and β chains are selectively substituted with mesoheme. Hb function is unaffected by the substitution, but the Soret absorbtion band shift in meso- relative to proto-heme permits chain-selective excitation of heme RR spectrum. The evolution of these spectra following HbCO photolysis show that geminate

recombination rates and yields are the same for the two chains, consistent with recent results with ^{15}N-heme isotopomer hybrids. The spectra also reveal systematic shifts in the deoxy-heme ν_4 and νFe-His RR bands, which are anti-correlated. These shifts are resolved for the successive intermediates in the protein structure, as previously determined from time-resolved UVRR spectra. Both chains show Fe-His bond compression in the immediate photoproduct, which relaxes during the formation of the first intermediate, R_{deoxy} (0.07 μs), in which the proximal F-helix is proposed to move away from the heme. Subsequently, the Fe-His bond weakens, more so for the α than the β chains. The weakening is gradual for the β chains, but abrupt for the α chains, coinciding with completion of the R-T quaternary transition, at 20 μs. Since the transition from fast- to slow-rebinding Hb also occurs at 20 μs, the drop in the α chain νFe-His supports the localization of ligation restraint to tension in the Fe-His bond, at least in the α-chains. The mechanism is more complex in the β chains.

2246-Pos Board B216

Production Of Bioactive NO From The Reaction Of Met-nitrite Hemoglobin With NO: Use Of Glassy Matices And Sol-gels

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There is growing evidence that nitrite-mediated reactions of Hb can generate bioactive forms of nitric oxide. The nitrite reductase reaction in which deoxy Hb reacts with nitrite has been studied from this perspective; however, this reaction generates NO under conditions where it can be easily scavenged. In contrast the proposed reaction of NO with nitrite bound to ferric heme derivatives to generate N_2O_3 is a much more promising mechanism. This follows because N_2O_3 can rapidly react with thiol containing peptides such as glutathione to produce S-NO derivatives. These species are likely to be the relatively long lived bioactive forms of NO that produce positive physiological effects in the vascular endothelium as occurs for blood substitutes derived from PEGylated Hb. We present results obtained using Hb samples encased in either glassy matrices or sol-gels that directly support this model. In particular there is spectroscopic evidence for the formation of a ferrous intermediate resulting from the direct reaction of NO with met-nitrite derivatives that precedes the formation of either ferrous NO or ferrous CO derivatives. This intermediate is attributable to a ferrous N_2O_3 complex.

2247-Pos Board B217

Novel Catalytic Antioxidative Activity of Nitroxide Radicals in the Heme/ H2O2 system

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Deleterious reactive oxygen species (ROS), are involved in cellular damage, dysfunction and diseases. Cell-permeable stable nitroxide radicals ($RNO^{\cdot}$), effectively protect against ROS-induced injury in cell-free, cellular, isolated organs models as well as in whole animals. Unlike common antioxidants, which operate stoichiometrically, and are progressively depleted particularly under oxidative stress, $RNO^{\cdot}$ can provide cytoprotection even at extremely low concentrations suggesting a catalytic mechanism. $RNO^{\cdot}$ antioxidative activity involves 1-electron exchange between its reduced (RNOH) and oxidized (RN^{+}=O) states, which allows its recycling. However, in spite of the significant therapeutic potential of nitroxides still, their protective mechanism(s) from ROS are not fully clear. A better mechanistic understanding of nitroxides activity in particular in the presence of heme proteins is essential for better selection of desired nitroxides, improvement of their efficacy, and minimizing potential adverse effects. Our objective is to elucidate the catalytic mechanism of nitroxides reaction under oxidative stress in the presence of heme proteins. We have studied $RNO^{\cdot}$ reaction in the heme/ H2O2 system and have found the kinetics to be far more complex than previously assumed. Our recent electron paramagnetic resonance (EPR) spectrometry results indicate transient oscillatory changes in [$RNO^{\cdot}$] during the catalytic dismutation of H2O2 and detoxification of the deleterious oxidized heme species such as heme protein radical and ferryl. Our results suggest that a key element in the catalytic antioxidative activity of nitroxides in the presence of heme/ H2O2 is a Belousov-Zhabotinsky like reaction mechanism, involving two reaction pathways (possibly ionic and radical).

2248-Pos Board B218

Conformational Changes Of Ferricytochrome c Induced by pH and Temperature

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This investigation is a comparison between the native and non-native states of ferricytochrome c as it is adopted between a pH of 1 to 13 and temperature of 278K to 353K. Visible electronic circular dichroism [ECD] and absorption spectroscopy were employed to probe the changes occurring at low ionic strength within the heme environment. The data presented is of the Soret band with the Q-band data and charge transfer band profile currently being gathered. At pH 2, state I, shows the emergence of a negative band, which is believed to represent the dissociation of both ligands from the heme. At this pH as the protein becomes unfolded and the iron is in a high spin state (Dyson, H, J.Biol.Chem, 1982), as the protein environment was acidified there was an emergence of a Cotton band in the CD spectra, also the intensity of the bands decreased, starting at pH 4. Approaching state III, as the iron enters a low spin state, there is a stronger couplet that emerges reflecting band splitting which is predominantly caused by a combination of electronic and vibronic perturbations (Reinhard Schweitzer-Stenner, J. Phys. Chem. B, 2008) and is maintained below 343K. This is suggesting that there is a conformational transition from the native state, into a thermally activated intermediate state, affecting the internal electric field causing moderate rearrangements of the heme, until it enters its thermally unfolded state. This unfolded state of the protein consistently becomes populated at higher temperatures across the pH range. This couplet remains into pH 9 which could reflect an intermediate transition of state III into state IV, moving to more alkaline states this couplet disappears. This study has overall shown significant heterogeneity of the protein throughout the pH range.

2249-Pos Board B219

Investigation Of The Role Of Neuroglobins In Globin Redox Reactions

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Neuroglobin (Ngb) is a recently identified member of the globins family. In the absence of external ligands, it is hexa-coordinated with a bis-hystidil heme at physiological conditions. This novel protein may protect the cell from hypoxia, since the protein is expressed whereas there is a raise in ROS (reactive oxygen species), typical of this condition. Unlike other hemoproteins such as Hemoglobins (Hbs) and Myoglobins (Mbs), Ngbs are not oxidized by hydrogen peroxyde (H_2O_2). In this work we have investigated by UV-Vis spectroscopy the influence of mouse ferric Ngb on the redox reactions between human ferrous and ferric Hb with H_2O_2 , a former of ROS species. The reaction of H_2O_2 with ferrous Hb produces the oxidation of the heme to the ferric metaHb (Fe^{+3}) state and X-ferryl-Hb (Fe^{+4}) state; the latter state is an intermediate species which reacts with Hb-O_2 (Fe^{+2}) exchanging electrons to produce metaHb. We show in this work that the amount of H_2O_2 required to induce the formation of ferryl and met-Hb, and from there to oxoferryl species, increases in the presence of Ngb. We have studied the effect of other known ROS scavengers, such as superoxide-dismutase and catalase, to compare with that observed in the presence of Ngb. Taken together the results of these studies suggest that Ngb might participate in the redox reactions, probably acting as a protector of globins from ROS. Further studies are in course to elucidade the mechanism of these reactions.

2250-Pos Board B220

Conformational Heterogeneity of Cytochrome c Probed by Resonance Raman Spectroscopy as a function of pH and Temperature

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The oxidized state of cytochrome c is a subject of continuous interest due to the multitude of conformations the protein adopts. Despite numerous studies, native and non-native states of ferricytochrome c have not been comprehensively analyzed regarding the influence of solvent conditions on structure, function, and thermodynamic equilibrium. Compared to the oxidized state, the reduced state of cytochrome c is rather stable since it adopts one conformation over a broad pH (2-12) and temperature (~100°C) range. In the current study, we have analyzed the high frequency (1200-1800cm^{-1}) Soret and Q-band resonance Raman spectra of oxidized and reduced horse heart cytochrome c (hhc) in terms of depolarization ratios and normalized Raman intensities, as a function of increasing pH and temperature. Initial analysis of our data collected for the Soret band resonance indicates that the depolarization ratios of A_{1g} modes ν_2, ν_3, and ν_4 of the native state III and the alkaline state IV are practically identical. They deviate from the respective D_{4h}-value, indicating that B_{1g} (triclinic) or B_{2g} (rhombic) type distortions affect the Raman tensor. For state III, the depolarization ratios of B_{1g} modes ν_{10}, ν_{11}, and ν_{13} deviate substantially from the expectation value indicating that these modes are affected by a large B_{1g} type distortion. The alkaline III-> IV transition moves the depolarization ratios of these B_{1g} modes closer to their D_{4h}-value of 0.75, indicating a substantial decrease of the rhombic B_{1g}-type deformation. Data from pH 12, which favor the population of the V-state, suggest a substantial increase of rhombic deformations, apparently caused by the replacement of a lysine by a hydroxyl ligand. Currently we are analyzing the depolarization ratios for the Q band resonance and the high temperature data for both resonances.